Supplementary Tables For:

Title: Structure of the Core of the Type Three Secretion System Export Apparatus

Authors: Lucas Kuhlen[1,2]†, Patrizia Abrusci[1]†, Steven Johnson[1]†, Joseph Gault[2], Justin Deme[1,3], Joseph Caesar[1,3], Tobias Dietsche[4], Mehari Tesfazgi Mebrhatu[4], Tariq Ganief[5], Boris Macek[5], Samuel Wagner[4,6], Carol V. Robinson[2] & Susan M. Lea[1,3*]

Affiliations:

[1]Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

[2]Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, UK.

[3]Central Oxford Structural Microscopy and Imaging Centre, University of Oxford, South Parks Road, Oxford OX1 3RE.

[4] Section of Cellular and Molecular Microbiology, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University of Tübingen, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany

[5] Proteome Center Tübingen, University of Tübingen, Auf der Morgenstelle 15, 72076 Tübingen, Germany

[6] German Center for Infection Research, Partner-site Tübingen, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany

*Correspondence to: Susan M. Lea, susan.lea@path.ox.ac.uk.

†These authors made equal contributions to the work presented herein.

Protein Complex	Fig 1 Panel	Deconvoluted Mass (Da)	Theoretical Mass (Da)	Difference (Da)	Assignment
Flagellum PQR in DDM	B (left)	155961	155917	44	P_5R_1
		165552	165534	18	$P_5Q_1R_1$
Flagellum PQR in LMNG	C (left)	196976	196959	17	$P_5Q_4R_1$
		206943	206909	34	$P_5Q_5R_1$
Injectisome PQR in DDM	B (right)	153825	153791	34	P_5R_1
		163274	163248	26	$P_5Q_1R_1$
		172725	172705	20	$P_5Q_2R_1$
Injectisome PQR in LMNG	C (right)	153795	153791	4	P_5R_1
		163245	163248	-3	$P_5Q_1R_1$
		172710	172705	5	$P_5Q_2R_1$
		182175	182163	12	$P_5Q_3R_1$

Protein Complex	Monomer Mass Used in Calculation (Da)	
Flagellum PQR in DDM *S.* Typhimurium	24567	P NterFormyl (cleavage site confirmed as Ala20)
	9618	Q 50% NterFormyl (ratio from dissociated monomer)
	33079	R NterFormyl
Flagellum PQR in LMNG *Pseudomonas savastanoi*	24964	P
	9950	Q NterFormyl
	32338	R (mass calculated from sequence)
Injectisome PQR in DDM & LMNG *Shigella flexneri*	24228	P NterFormyl
	9457	Q NterFormyl
	32652	R NterFormyl

Table S1. Masses of protein complexes measured in Fig 1. and assignment based on comparison with theoretical masses. The site of signal peptide cleavage for FliP, and N terminal modification for all other species was determined by high energy dissociation of the intact complexes and mass measurement of individual subunits by native mass spectrometry. Theoretical masses were then calculated from the sequence taking into account any observed posttranslational modifications.

	P1 (A)	P2 (B)	P3 (C)	P4 (D)	P5 (E)	R (F)	Q1 (G)	Q2 (H)	Q3 (I)	Q4 (J)
P1 (A)		1843.6	11.6			1786.1	1115.8	96.6		
P2 (B)	1843.6		1871	9.9		50.5	163.1	1169.9	117.3	
P3 (C)	11.6	1871		1750.1		81.5	46.5	166.6	1105.6	119.9
P4 (D)		9.9	1750.1		1548.2	206.1		37.6	162.3	1092.8
P5 (E)				1548.2		2142.5			41.4	162.8
R (F)	1786.1	50.5	81.5	206.1	2142.5		903.4			8.7
Q1 (G)	1115.8	163.1	46.5			903.4		489.2		
Q2 (H)	96.6	1169.9	166.6	37.6			489.2		467.7	
Q3 (I)		117.3	1105.6	162.3	41.4			467.7		505.9
Q4 (J)			119.9	1092.8	162.8	8.7			505.9	

Table S2. Protein surface area (Å^2) buried on each chain in assembly of $P_5Q_4R_1$export gate complex as assessed using PISA

Contacts between PQR and B	
Residue in R	Residue in B
L188	A182
T192	A186
A200	T160
L199	V190

Contacts between PQR and the rod	
Residue in P	Residue in FliE
V42	G85
L58	V99
Residue in P	Residue in FlgB
L45	V133
T44	V133

Table S3. Co-evolutionary contacts between PQR, B and FliE and FlgB determined using Gremlin [22].

Name	Sequence
gib_pT12_PrgK_f	GAAATTCAGG AGGAATTCAC CATGATTCGT CGATATCTAT ATACTTTTC
gib_pT12_prgK_r2	CTTCTCTCAT CCGCCAAAAC AGCCAAGCTT CATTCATTTG ACGATTTCGC CTTATC
gib_uni_pT12_f	AGCTTGGCTGTTTTGGCGGATG
gib_uni_pT12_r	GGTGAATTCCTCCTGAATTTC
prgK_E138X_QC_f	CCATATTAGTTATGATATTGATGCTGGTTAGAATGGCCGCCCGCCAAA AC
prgK_E138X_QC_r	GTTTTGGCGGGCGGCCATTCTAACCAGCATCAATATCATAACTAATATG G
pT12_f	GAAAACCTGTACTTCCAGGGTC
pT12_r	TGGTGAATTCCTCCTGAATTTC
FliO_SALTY_f	CAGGAGGAATTCACCATGAAGACAGAAGCCACGGTTTCTC
FliR_SALTY_r	GAAGTACAGGTTTTCTGGGTTATTATTTATCGGCATCTCG
FliO_PSESH_f	TCAGGAGGAATTCACCATGAGGCGCTGGCTAAGTGTTCTG
FliR_PSESH_r	CCCTGGAAGTACAGGTTTTCACGAGCCCTCACCATATCGC
Spa24_f	GACTGGTCGTAATGAAGATGGTAAAGGAGTAATCTCATGCTG
Spa29_r	GAAGTACAGGTTTTCTCTAACAAATAGATTTGTGAAAAATTTATGTTC
pBAD_f	AGCGCCGTCGACCATCATC
pBAD_r	CATGGTTAATTCCTCCTGTTAGCCC
FlhB_f	GAGGAATTAACCGTGGCAGAAGAGAGCGACG
FlhA_r	GATGATGGTCGACGGCGCTTTTTCCTCCAATGGTCGCCG

Table S4. Primers used in this study

Escherichia coli	**Description**	**Reference**	**Construction note**
MT56		Baumgarten, T., Schlegel, S., Wagner, S., Löw, M., Eriksson, J., Bonde, I., et al. (2017). Isolation and characterization of the *E. coli* membrane protein production strain Mutant56(DE3). Scientific Reports, 7, 45089. http://doi.org/10.1038/srep45089	

Salmonella	**Description**	**Reference**	**Construction note**
MIB3147	SL1344, Δ*prgK*, $SpaP^{FLAG}$, *flhD::tet*	this study	Made by allelic exchange using the following suicide plasmid: pSB3537, pMIB5169
MIB3148	SL1344, Δ*prgK*, $SpaR^{FLAG}$, *flhD::tet*	this study	Made by allelic exchange using the following suicide plasmid: pSB3539, pMIB5169
MIB3118	SL1344, $InvC_{K165E}$, *flhD::tet*	this study	Made by allelic exchange using the following suicide plasmid: pMIB5264

Plasmids	**Description**	**Reference**	**Construction note**
pSB3537	pSB890 $SpaP^{FLAG}$	Wagner, S., Königsmaier, L., Lara-Tejero, M., Lefebre, M., Marlovits, T. C., & Galán, J. E. (2010). Organization and coordinated assembly of the type III secretion export apparatus. Proceedings of the National Academy of Sciences of the United States of America, 107(41), 17745–17750. http://doi.org/10.1073/pnas.1008053107	
pSB3539	pSB890 $SpaR^{FLAG}$	Wagner, S., Königsmaier, L., Lara-Tejero, M., Lefebre, M., Marlovits, T. C., & Galán, J. E. (2010). Organization and coordinated assembly of the type III secretion export apparatus. Proceedings of the National Academy of Sciences of the United States of America, 107(41), 17745–17750. http://doi.org/10.1073/pnas.1008053107	
pMIB5169	pSB890 Δ*prgK*	Dietsche, T., Tesfazgi Mebrhatu, M., Brunner, M. J., Abrusci, P., Yan, J., Franz-Wachtel, M., et al. (2016). Structural and functional characterization of the bacterial type III secretion export apparatus. PLoS Pathogens, 12(12), e1006071. http://doi.org/10.1371/journal.ppat.1006071	
pMIB5264	pSB890 $InvC_{K165E}$	Dietsche, T., Tesfazgi Mebrhatu, M., Brunner, M. J., Abrusci, P., Yan, J., Franz-Wachtel, M., et al. (2016). Structural and functional characterization of the bacterial type III secretion export apparatus. PLoS Pathogens, 12(12), e1006071. http://doi.org/10.1371/journal.ppat.1006071	Made by site-directed mutagenesis using primers prgK_E138X_QC_f and prgK_E138X_QC_r.
pMIB6110	pT10 *prgK*	this study	Made by Gibson assembly of PCR products of the following two primer/template pairs: 1. gib_pT12_prgK_f + gib_pT12_prgK_r2 from SL1344 chromosomal DNA; 2. gib_uni_pT12_f + gib_uni_pT12_r from pSB3398 (pT10). Mutated the wt TAG stop codon of *prgK* to a TGA stop codon.
pMIB6679	pT10 $PrgK_{E138pBpa}$	this study	Made by site-directed mutagenesis using primers prgK_E138X_QC_f and prgK_E138X_QC_r.
pSB3292	pBAD24 *hilA*	Lara-Tejero, M., Kato, J., Wagner, S., Liu, X., & Galán, J. E. (2011). A sorting platform determines the order of protein secretion in bacterial type III systems. Science (New York, NY), 331(6021), 1188–1191. http://doi.org/10.1126/science.1201476	

pSup-pBpa		Ryu, Y., & Schultz, P. G. (2006). Efficient incorporation of unnatural amino acids into proteins in Escherichia coli. Nature Methods, 3(4), 263–265. http://doi.org/10.1038/nmeth864	
pMIB5689	pT12, SpaPQR^{TEV2xStrepII}	Dietsche, T., Tesfazgi Mebrhatu, M., Brunner, M. J., Abrusci, P., Yan, J., Franz-Wachtel, M., et al. (2016). Structural and functional characterization of the bacterial type III secretion export apparatus. PLoS Pathogens, 12(12), e1006071. http://doi.org/10.1371/journal.ppat.1006071	
pT12_FliOPQR_SALTY	*S.* Typhimurium FliOPQR^{TEV2xStrepII}	this study	Made by Gibson assembly of PCR products of the following two primer/template pairs: 1. pT12_f + pT12_r from pMIB5689; 2. FliO_SALTY_f + FliR_SALTY_r from *S.* Typhimurium genomic DNA.
pT12_FliOPQR_PSESH	*P. savastanoi* FliOPQR^{TEV2xStrepII}	this study	Made by Gibson assembly of PCR products of the following two primer/template pairs: 1. pT12_f + pT12_r from pMIB5689; 2. FliO_PSESH_f + FliR_PSESH_r from *P. savastanoi* genomic DNA (DSM-21482).
pT12_Spa24929	*S. flexneri* SctRST^{TEV2xStrepII}	this study	Made by Gibson assembly of PCR products of the following two primer/template pairs: 1. pT12_f + pT12_r from pMIB5689; 2. Spa24_f + Spa29_r from the *S. flexneri* virulence plasmid pWR100.
pBAD_FlhBA	*S.* Typhimurium FlhBA^{His}	this study	Made by Gibson assembly of PCR products of the following two primer/template pairs: 1. pBAD_f + pBAD_r from pBAD; 2. FlhB_f + FlhA_r from *S.* Typhimurium genomic DNA.

Table S5. Strains and plasmids used